

# Fumigation toxicity of volatile natural and synthetic cyanohydrins to stored-product pests and activity as soil fumigants

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**Abstract:** Insecticidal fumigation toxicity of natural and synthetic cyanohydrins was evaluated with four stored-product pests: the lesser grain borer, *Rhyzopertha dominica* (F), the red flour beetle, *Tribolium castaneum* Herbst, the saw-toothed grain beetle *Oryzaephilus surinamensis* L, the maize weevil, *Sitophilus zeamais* (Motsch) and the house fly, *Musca domestica* L. The fumigation LC<sub>50</sub> values were calculated by probit analysis. For house flies, all but one of the cyanohydrins tested were more potent than 1,3-dichloropropene (Telone®). Three were as efficacious as chloropicrin. For the lesser grain borer, all cyanohydrins tested were more insecticidal than dichloropropene, and all but one were more potent than chloropicrin. Four were as insecticidal as dichlorvos. The acetate of 1-cyano-1-hydroxy-2-propene (CHP-ace) was also tested in soil for antifungal and antibacterial activity, and inhibition of weed seed germination. CHP-ace reduced the total soil bacterial and fungal counts significantly, and was effective in inhibiting the germination of weed seeds in soil, indicating a broad spectrum of activity as a soil fumigant.

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**Keywords:** fumigation; cyanohydrin; stored product pests; insecticide; methyl bromide; chloropicrin; dichlorvos; dichloropropene

## 1 INTRODUCTION

Secondary plant metabolites are useful for defense against herbivores. Many food and feed plants have been shown to synthesize cyanogenic compounds which can decompose to produce hydrocyanic acid (HCN) as a main source of plant defense, which acts as a toxicant or feeding deterrent to herbivores.<sup>1</sup> Cassava, lima beans, peas, almonds, white clover, bamboo and flax all produce cyanogenic compounds. Some cyanogenic plants are grown and used for starch, protein, oil or fiber sources, and as spices or crude drugs.<sup>2</sup> The cyanogenic glycosides are biosynthesized by the plants from aromatic or branched-chain amino acids.<sup>3</sup> The biochemical system for the formation of free HCN, cyanogenesis, is associated with a cyanogenic glycoside that is hydrolyzed by a  $\beta$ -glycosidase to produce a hydroxynitrile (cyanohydrin), which then decomposes to a carbonyl compound and HCN.<sup>4</sup> As a non-selective respiratory inhibitor, hydrogen cyanide (hydrocyanic acid) has been used for many years as a fumigant for insects, inhibiting cytochrome  $a_3$  in the mitochondrial electron-transport system.<sup>5</sup>

Although there are many commercial fumigants, such as methyl bromide, dichlorvos, chloropicrin and

phosphine, regulatory problems exist with several current fumigants. The US Environmental Protection Agency (EPA) has been restricting the use of methyl bromide, an ozone depleter, and dichlorvos, a suspected carcinogen.<sup>6</sup> As a result of their toxicity, methyl bromide, phosphine, dichloropropene, chloropicrin and dichlorvos use is restricted to licensed commercial applicators.<sup>7–9</sup>

In our study, we conducted tests on the fumigation toxicity of several natural and synthetic volatile cyanohydrins. This research compares the effectiveness of the cyanohydrins against five species of insect pests. Their potency is also compared with some commercial fumigants. Various concentrations of 1-cyano-1-hydroxy-2-propene acetate (CHP-ace, acetoxybutenenitrile) were tested as a general soil fumigant in this study. Specifically, antibacterial and antifungal activity, as well as inhibition of weed seed germination, were measured in treated soils. Methyl bromide fumigation had been performed earlier on soil in this laboratory, and the effect on micro-organisms was compared with this. As an additional comparison, the effect of chloropicrin and 1,3-dichloropropene (active ingredient in Telone) on weeds was also determined.

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(Received 22 July 2002; revised version received 16 July 2003; accepted 14 August 2003)

Published online 22 March 2004

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jar were calculated using an assumption that all of the compound volatilized off the filter paper.

### 2.2.2 Fumigation of stored product insects

Ten adult beetles of each species (*Rhyzopertha dominica* (F), *Tribolium castaneum* Herbst, *Oryzaephilus surinamensis* L, or *Sitophilus zeamais* (Motsch)), with 1 g of grain for food, were placed into a glass tube (1.5 cm × 5 cm) fitted with a metal screen, which was secured by paraffin film on each end. This tube was suspended in a 490-ml glass mason jar with a folded piece of 9-cm Whatman #4 filter paper. Each test compound was dissolved in corn oil, and 100 µl of the appropriate solution was applied to the filter paper. The jar was securely capped, and mortalities were recorded after 24 h. This test was replicated three times.

## 2.3 Soil fumigant activity

### 2.3.1 Soil bacteria and fungi study

The soil was collected from an agricultural field plot in Ames, IA. The soil type was a loam (sand 44%, silt 32%, and clay 24%). The pH was 7.0, and the organic matter content was 2.7%. The soil was incubated in a Percival environmental growth chamber (Percival Co., Boone, Iowa). The photoperiod was 12:12 h light:dark and the temperature was 24 (±1) °C inside the chamber. The six treatments were: blank control, solvent control (0.5 ml acetone), and solutions of CHP-ace in acetone (0.5 ml) at concentrations of 10, 100, 1000 and 10 000 mg liter<sup>-1</sup>, giving 0.5, 5.50 and 500 µg g<sup>-1</sup> soil, respectively. Each treatment had three replications. The soil was incubated on a laboratory bench for 12 h before the treatments were applied. Ten grams (dry-weight) soil, at a moisture content of approximately 100% holding capacity, were then put into a sterilized 260-ml French square bottle, and the appropriate solution applied. After the treatment, the soil was placed in the incubator for an additional 8 h. Sterilized phosphate buffer (90 ml) was then added to each bottle, and the bottles were shaken for approximately 30 min. Serial dilutions were then carried out, ie, the solution was progressively diluted by factors of 10. Three or four replicates were used for each concentration of each compound. 1,3-Dichloropropene and chloropicrin were used as the commercial standards for comparison. 1,3-Dichloropropene is known to have fumigant activity against nematodes and weeds similar to that of methyl bromide.<sup>13</sup> A microdrop plate-count technique was then used to count the number of bacteria and fungi. This method generally involves applying the solutions to an agar medium in Petri dishes. The number of colony-forming units can then be calculated per gram of soil. This method provides a means for determining an estimate of microbial activity in soil.<sup>14</sup> Such results provide a general response of soil microbes to tested compounds in a dose-response assay.

### 2.3.2 Weed study

In the case of the weed study, three treatments were used initially: a solvent control (0.5 ml acetone), CHP-ace (1000 mg liter<sup>-1</sup>; 0.5 ml) for a final concentration of 10 µg g<sup>-1</sup> soil, and CHP-ace (10 000 mg liter<sup>-1</sup>; 0.5 ml) to give 100 µg g<sup>-1</sup> soil. Later tests also included 1-ml treatments of 1000 mg liter<sup>-1</sup> (10 µg g<sup>-1</sup> soil) and 10 000 mg liter<sup>-1</sup> (100 µg g<sup>-1</sup> soil) of chloropicrin and dichloropropene as noted above. In all cases, 50 g of soil were used in each replication. The soil was put in glass mason jars, and the jars were kept in the greenhouse for 3 days before the chemical treatments were applied to the surface of the soils. Three replications were done in all instances. For the CHP-ace and 0.5 ml solvent control tests, a count of germinated weeds was made at 3 and 24 days after treatment. For the chloropicrin and dichloropropene tests, the mason jars were kept in the greenhouse for 4 days before the chemical treatments were applied to the surface of the soils. Counts were made 10 days after treatment.

### 2.3.3 Microbial respiration study

In the case of the microbial respiration study, 20 g (dry weight) of soil were placed in stoppered, 250-ml glass jars, and soil moisture was adjusted to -33 kPa approximately of field-capacity. Methyl bromide was applied at concentrations of 350 µg g<sup>-1</sup> (ie 350 ppm) and 2733 µg g<sup>-1</sup> as noted above. The soils were incubated in the dark at 25 °C. A complete description of the test set-up and results was given by Rice *et al.*<sup>15</sup> The method used to monitor microbial activity in the soil was the measurement of carbon dioxide generated per time interval. Specifically, carbon dioxide efflux was measured at 24-h intervals after an initial 48-h fumigation period. The sample headspace was purged with moist, carbon dioxide-free air and was analyzed using an infrared gas analyzer. Microbial respiration in the methyl bromide-fumigated and untreated samples was compared. 'Microbial activity' refers to normal respiration, metabolism and growth of soil micro-organisms. In this case, microbial activity refers to 'aerobic' activity, ie use of oxygen and carbon sources for energy and growth, producing carbon dioxide and water as waste products. 'Normal' microbial activity is determined in any given experiment by the activity in the untreated control soil. 'Slightly depressed' activity refers to activity that is 50% or more of the control activity, such that carbon dioxide production is 50% or more of the production in the control. 'Severely depressed' microbial respiration activity refers to activity that is less than 20% of control activity.

### 2.3.4 Statistical methods

All of the fumigation LC<sub>50</sub> values determined in this study were calculated using Proc Probit on SAS.<sup>16</sup> Comparisons of compounds' toxicities in the Results section (using Tables 1 and 2) are based on the 95% fiducial limits (95% FL); LC<sub>50</sub> values that have overlapping FLs are considered to be comparable; if

**Table 2.** Fumigation LC<sub>50</sub> values ( $\mu\text{g ml}^{-1}$ ) of cyanohydrins and derivatives

Compounds	<i>T. castaneum</i>	95% FL <sup>a</sup>	<i>O. surinamensis</i>	95% FL <sup>a</sup>	<i>S. zeamais</i>	95% FL <sup>a</sup>
CHP	12.1	10.9, 13.1	0.5	0.4, 0.6	2.5	2.3, 2.8
CHP-ace	22.5	22.2, 27.4	0.7	0.6, 0.8	2.7	2.5, 3.0
CHP-me	4.8	4.2, 5.4	2.1	1.9, 2.3	2.6	2.4, 2.9
CPC-CNOH	36	31.4, 41.6	0.5	0.4, 0.6	1.3	1.1, 1.4
CPM-CNOH	32	23.7, 39.6	0.2	0.2, 0.3	0.7	0.6, 0.8

<sup>a</sup> 95% fiducial limits.**Table 3.** Total bacterial count in soil 2 days after application of CHP-ace

Treatment (CHP-ace)	Average (CFU g <sup>-1</sup> dry wt)	SD
Blank control	$4.7 \times 10^6$	$1.8 \times 10^6$
Solvent control	$3.2 \times 10^6$	$0.2 \times 10^6$
0.5 $\mu\text{g g}^{-1}$	$4.6 \times 10^6$	$2.6 \times 10^6$
5 $\mu\text{g g}^{-1}$	$16.2 \times 10^6$	$3.2 \times 10^6$
50 $\mu\text{g g}^{-1}$	$0.2 \times 10^6$	$0.02 \times 10^6$
500 $\mu\text{g g}^{-1}$	0.00	0.00

**Table 4.** Total fungal count in soil 2 days after application of CHP-ace

Treatment (CHP-ace)	Average (CFU g <sup>-1</sup> dry wt)	SD
Blank control	$1.9 \times 10^4$	$0.4 \times 10^4$
Solvent control	$1.4 \times 10^4$	$0.4 \times 10^4$
0.5 $\mu\text{g g}^{-1}$	$2.0 \times 10^4$	$0.5 \times 10^4$
5 $\mu\text{g g}^{-1}$	$0.5 \times 10^4$	$0.4 \times 10^4$
50 $\mu\text{g g}^{-1}$	0.00	0.00
500 $\mu\text{g g}^{-1}$	0.00	0.00

**Table 5.** Number of germinated weed seeds 3 days after application of CHP-ace

Treatment	Number of germinated weeds for replication				P-value
	1	2	3	Av	
Solvent control	6	8	11	8.3	0.0006
10 $\mu\text{g g}^{-1}$	0	0	0	0	
100 $\mu\text{g g}^{-1}$	0	0	0	0	

**Table 6.** Number of germinated weed seeds 24 days after application of CHP-ace

Treatment	Number of germinated weeds for replication				P-value
	1	2	3	Av	
Solvent control	10	20	27	19	0.0048
10 $\mu\text{g g}^{-1}$	0	0	0	0	
100 $\mu\text{g g}^{-1}$	0	0	0	0	

seeds in soil, and also show the statistically significant difference between control and each treatment. It was also observed that CHP-ace at both the 10 and 100  $\mu\text{g g}^{-1}$  concentrations can also kill post-emergent weeds. Tables 7 and 8 show the average number of germinated weeds following treatment

**Table 7.** Number of germinated weed seeds 10 days after application of chloropicrin

Treatment	Number of germinated weeds for replication				P-value
	1	2	3	Av	
Solvent control	0	12	3	5	0.2963
10 $\mu\text{g g}^{-1}$	0	3	0	1	
100 $\mu\text{g g}^{-1}$	0	0	0	0	

**Table 8.** Number of germinated weed seeds 10 days after application of 1,3-dichloropropene

Treatment	Number of germinated weeds for replication				P-value
	1	2	3	Av	
Solvent control	0	12	3	5	0.3313
10 $\mu\text{g g}^{-1}$	4	4	8	5.3	
100 $\mu\text{g g}^{-1}$	0	2	0	0.7	

**Table 9.** Severity and number of days microbial activity was depressed by treatment of soil with fumigant (data from Rice et al.<sup>15</sup>)

Treatment	Severity	Days
Control	Normal activity	0
350 $\mu\text{g g}^{-1}$ MeBr	Slightly depressed	4
2733 $\mu\text{g g}^{-1}$ MeBr	Severely depressed	24
250 $\mu\text{g g}^{-1}$ CHP-ace	No bacteria or fungal colonies formed after 2 days	

with 1 ml of a solvent control and two different concentrations of chloropicrin and dichloropropene, respectively, for three different replications after 10 days, respectively. Tables 7 and 8 show that both chloropicrin and dichloropropene are effective in inhibiting the germination of weed seeds in soil, but less effectively than CHP-ace in terms of the difference in the numbers of germinated weed seeds between control and each treatment. It was also observed during testing that dichloropropene and/or chloropicrin, at both 10 and 100  $\mu\text{g g}^{-1}$  concentrations, can also kill post-emergent weeds. Although germinating weeds were not counted on the same days for all tests, and variability was high in the chloropicrin and dichloropropene tests, the compounds in the present study showed effectiveness comparable to chloropicrin and dichloropropene.

Table 9 shows the severity and number of days for which microbial activity was depressed by treatment